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Accelerated Communication

IBOGAINE POSSESSES A SELECTIVE AFFINITY FOR σ_2 RECEPTORSRobert H. Mach,^{1,2} Cynthia R. Smith,¹ and Steven R. Childers²

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Abstract. The alkaloid ibogaine is potentially useful to reduce craving for several drugs of abuse, but its mechanism of action is not known. In the current study, in vitro studies were conducted in order to determine the affinity of ibogaine for sigma receptors. Our results indicate that ibogaine has a relatively high affinity for σ_2 receptors ($K_i = 90.4$ and 250 nM) and a significantly lower affinity for σ_1 receptors ($K_i = 9310$ nM). These data suggest that ibogaine may have a higher affinity at σ_2 receptors than any other known CNS receptor. Its low affinity for σ_1 receptors also suggests that ibogaine may be a suitable lead compound for structure-activity relationship studies aimed at developing σ_2 -selective ligands.

Key Words: ibogaine, sigma receptors, substance abuse

Introduction

Ibogaine (EndabuseTM) is an indole alkaloid isolated from the root of the African shrub *Tabernanthe iboga*. In low doses it has been shown to function as a CNS stimulant, whereas at higher doses ibogaine is known to produce both hallucinogenic and tremorigenic effects (1,2). A number of anecdotal reports have suggested that ibogaine may be effective in reducing the craving for heroin, cocaine, and amphetamines in individuals known to abuse these substances (3). These observations have also been supported by research using animal models of substance abuse, with ibogaine demonstrating activity in reducing amphetamine-induced, morphine-induced, and cocaine-induced locomotor activity in rats (4) and mice (5,6), as well as morphine and cocaine self-administration in rats (1,7-9). In spite of the positive attributes of ibogaine in animal self-administration studies, there is some concern regarding the safety of this approach in the treatment of substance abuse since there is evidence suggesting that ibogaine may be neurotoxic at levels somewhat higher than the behaviorally-active doses. This includes a decrease in Purkinje cells and an activation of glial cells in the vermis of the cerebellum, a region not identified as playing an active role in mediating the reinforcing properties of abused drugs (3,10,11). Although much attention has been focused on identifying its primary site of action, little is known regarding the mechanism of action of the therapeutic properties of ibogaine or the neurochemical basis of its toxicity in the CNS.

In vitro binding studies have shown ibogaine to possess a low (>100 μ M) affinity for several neurotransmitter receptors including adrenergic (α_1 , α_2 , and β_1), cannabinoid, dopaminergic (D_1 , D_2), GABAergic (GABA_A, benzodiazepine), cholinergic (both nicotinic and muscarinic), serotonergic (5-HT_{1A-D}, 5-HT₂, 5-HT₃) and opiate (μ , δ) receptors (12). Although ibogaine possesses a low micromolar affinity for the dopamine transporter ($IC_{50} \sim 1.5$ μ M vs. [³H]WIN 35,248 in mouse striatum) (5), κ opiate receptors ($K_i \sim 2$ μ M vs. [³H]U69,593 binding to bovine cortex) (12), and the MK-801 binding site of the NMDA receptor-channel complex ($K_i \sim 1$ μ M) (13), it is very likely that another site of action may be responsible for the reduced drug craving properties of ibogaine. Until a primary site of action is identified, a structure-activity relationship

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study aimed at improving the therapeutic index of ibogaine is likely to be an arduous task.

The purpose of the current study was to measure the affinity of ibogaine for σ_1 and σ_2 receptors. Our interest in the sigma binding properties of ibogaine stems from previous reports showing that ibogaine and sigma ligands both block the locomotor effects of cocaine (5,14). In addition, a recent self-administration study in rats presented evidence showing ibogaine pretreatment resulted in considerable abnormal motor effects (9). Similar motor abnormalities have been reported when sigma ligands are microinjected into the red nucleus of rats (15). The results of our studies clearly indicate that ibogaine has a relatively high affinity for σ_2 but not σ_1 receptors, and suggests that the behavioral effects of ibogaine may be mediated through an interaction with the σ_2 receptor.

Methods

Drugs. Ibogaine was provided by the National Institute on Drug Abuse. Haloperidol was purchased from Aldrich Chemical Co., Milwaukee WI; haloperidol metabolite II and (+)-pentazocine were purchased from Research Biochemicals International, Natick, MA.

Sigma Receptor Binding. Sigma-1 binding sites were labeled with the σ_1 -selective radioligand, [3 H](+)-pentazocine (Dupont-NEN) in guinea pig brain membranes (Rockland Biological) according to published procedures (15,16). Sigma-2 sites were assayed in rat liver membranes, a rich source of these sites (15,16), with [3 H]DTG (Dupont-NEN) in the presence of (+)-pentazocine (100 nM). Sigma-2 sites were also assayed in guinea pig membranes with [3 H]DTG in the presence of (+)-pentazocine (100 nM).

Membrane Preparation. Crude P2 membranes were prepared from frozen guinea pig brains minus cerebellum. Brains were allowed to thaw slowly on ice before homogenization. Crude P2 membranes were also prepared from the livers of male Sprague-Dawley rats (300-350 g). Animals were sacrificed by decapitation and the livers removed and minced before homogenization. Tissue homogenization was carried out at 4°C in 10 mL/g tissue weight of 10 mM Tris-HCl/0.32 M sucrose, pH 7.4 using a Potter-Elvehjem tissue grinder. The crude homogenate was centrifuged for 10 min at 1000 g and the supernatant saved on ice. The pellet was resuspended in 2 mL/g tissue weight of ice-cold 10 mM Tris-HCl/0.32M sucrose, pH 7.4 by vortexing. After centrifuging at 1000 g for 10 min, the pellet was discarded and the supernatants were combined and centrifuged at 31,000 g for 15 min. The pellet was resuspended in 3 mL/g 10 mM Tris-HCl, pH 7.4 by vortexing, and the suspension was allowed to incubate at 25°C for 15 min. Following centrifugation at 31,000 g for 15 min, the pellet was resuspended by gentle homogenization to 1.53 mL/g in 10 mM Tris-HCl (pH 7.4) and aliquots were stored at -80°C until used. The protein concentration of the suspension was determined by the method of Bradford and generally ranged from 6-11 mg protein/mL.

σ_1 Binding Assay. Guinea pig brain membranes (100 μ g protein) were incubated with 3 nM [3 H](+)-pentazocine (31.6 Ci/mmol) in 50 mM Tris-HCl, pH 8.0 at 25°C for either 120 or 240 min. Test compounds were dissolved in ethanol then diluted in buffer for a total incubation volume of 0.5 mL. Assays were terminated by the addition of ice-cold 10 mM Tris-HCl, pH 8.0 followed by rapid filtration through Whatman GF/B glass fiber filters (presoaked in 0.5 % polyethylenimine) using a Brandel cell harvester (Gaithersburg, MD). Filters were washed twice with 5 mL of ice cold buffer. Nonspecific binding was determined in the presence of 10 μ M (+)-pentazocine. Liquid scintillation counting was carried out in EcoLite(+) (ICN Radiochemicals; Costa Mesa, CA) using a Beckman LS 6000IC spectrometer with a counting efficiency of 50%. Typical counts were 70 dpm/ μ g protein for total binding, 6 dpm/ μ g protein for nonspecific binding, and 64 dpm/ μ g protein for specific binding.

σ_2 Binding Assay. Rat liver membranes (35 μ g of protein) or guinea pig brain membranes (360 μ g) were incubated with 3 nM [3 H]DTG (38.3 Ci/mmol) in the presence of 100 nM (+)-pentazocine to mask σ_1 sites. Incubations were carried out in 50 mM Tris-HCl, pH 8.0 for 120 min at 25°C in a total incubation volume of 0.5 mL. Assays were terminated by the addition of ice-cold 10 mM Tris-HCl, pH 8.0 followed by rapid filtration through Whatman GF/B glass fiber filters (presoaked in 0.5

% polyethylenimine) using a Brandel cell harvester (Gaithersburg, MD). Filters were then washed twice with 5 mL of ice cold buffer. Nonspecific binding was determined in the presence of 5 μ M DTG. Liquid scintillation counting was carried out in EcoLite(+) (ICN Radiochemicals; Costa Mesa, CA) using a Beckman LS 6000IC spectrometer with a counting efficiency of 50%. Typical counts for rat liver were 297 dpm/ μ g protein for total binding, 11 dpm/ μ g protein for nonspecific binding, and 286 dpm/ μ g protein for specific binding. Typical counts for guinea pig brain were 16 dpm/ μ g protein for total binding, 2 dpm/ μ g protein for nonspecific binding, and 14 dpm/ μ g protein for specific binding.

Data Analysis. The IC₅₀ values at sigma sites were determined in triplicate from non-linear regression of binding data as analyzed by JMP (SAS Institute; Cary, NC), using 5-10 concentrations of each compound. K_i values were calculated using the Cheng-Prusoff equation (17) and represent mean values $\pm$ SEM. All assays were done in triplicate unless otherwise noted. The K_d value used for [³H]DTG in rat liver was 17.9 nM and was 4.8 nM for [³H](+)-pentazocine in guinea pig brain (15,16). The K_d value for [³H]DTG in guinea pig brain was determined by separate Scatchard analyses to be 21.6 nM.

Results

Binding constants (K_i values) for the inhibition of [³H](+)-pentazocine to σ_1 receptors and [³H]DTG to σ_2 receptors are shown in Table I. Competition curves for the binding experiments using guinea pig brain membranes (σ_1) and rat liver membranes (σ_2) are also represented in Figure 1. In addition to ibogaine, haloperidol and haloperidol metabolite II (reduced haloperidol) were included in the binding assays as external standards. Both haloperidol and haloperidol metabolite II had a high affinity for σ_1 and σ_2 receptors, and the values obtained from the present studies are consistent with the reported values for these compounds (15,16,18). In contrast, ibogaine was found to have a low affinity for σ_1 receptors (K_i = 9310 nM) and a relatively high affinity for σ_2 receptors in rat liver (K_i = 90.4 nM). In order to confirm that the binding of ibogaine to σ_2 receptors in rat liver is equivalent to σ_2 receptors in brain, the assay was also repeated using guinea pig brain as the tissue source. The affinity of ibogaine to σ_2 sites in guinea pig brain was slightly lower than that observed in rat liver (K_i = 250 nM). This difference in σ_2 binding affinity of ibogaine may be due to the difference in species (rat vs. guinea pig) or tissue source (liver vs. brain) in the σ_2 membrane preparation. Similar differences in binding constants have been reported with σ_2 receptors obtained from different tissue sources within the same species (16). The σ_2 : σ_1 selectivity, which is the ratio of the K_i values for each site, was 103 (K_i for σ_1 /K_i for σ_2 (liver)) and 37.2 (K_i for σ_1 /K_i for σ_2 (brain)) for ibogaine. All three compounds had Hill slopes of $\sim$ 1.0 (data not shown), indicating the absence of positive or negative cooperativity of binding at each σ receptor.

Table I. Affinities of Ibogaine, Haloperidol, and Haloperidol Metabolite II for Sigma Receptors.

Compound	K _i [nM] (n = 3) ^a			
	σ_1 ^b	σ_2 (liver) ^c	σ_2 (brain) ^d	σ_2 : σ_1 Selectivity
ibogaine	9310 $\pm$ 630	90.4 $\pm$ 10.1 ^e	250 $\pm$ 39 ^f	103 ^g or 37.2 ^h
haloperidol	1.77 $\pm$ 0.09	21.8 $\pm$ 8.5	14.2 $\pm$ 3.2 ⁱ	0.08 ^g or 0.13 ^h
haloperidol metabolite II	5.65 $\pm$ 0.26	25.4 $\pm$ 3.3	1.31 $\pm$ 0.03	0.22 ^g or 4.30 ^h

^aMean $\pm$ SEM; ^bK_i for inhibiting the binding of [³H](+)-pentazocine to guinea pig brain membranes;

^cK_i for inhibiting the binding of [³H]DTG to rat liver membranes; ^dK_i for inhibiting the binding of [³H]DTG to guinea pig brain membranes; ^eN = 4; ^fN = 7; ^gK_i for σ_1 /K_i for σ_2 (liver); ^hK_i for σ_1 /K_i for σ_2 (brain).

Discussion

The goal of the current study was to determine if ibogaine possessed an affinity for either σ_1 or σ_2 receptors. The σ_1 assay was conducted using membranes isolated from guinea pig brain, a tissue previously shown to possess a high density of σ_1 receptors (15). The radioligand used was [3 H](+)-pentazocine, which has been reported to have a high affinity and high selectivity for σ_1 receptors (15,16). However, a recent study presented evidence suggesting that [3 H](+)-pentazocine labeled two sites in human cerebellum (19) with K_d values of 1.4 and 33.6 nM when the incubation period was extended to 4 hours. The binding profile of the high affinity site was shown to be identical with the σ_1 receptor, whereas the identity of the low affinity site was suggested to be the σ_2 receptor; other possibilities, such as the dopamine D_3 receptor, were also raised. In order to confirm that the

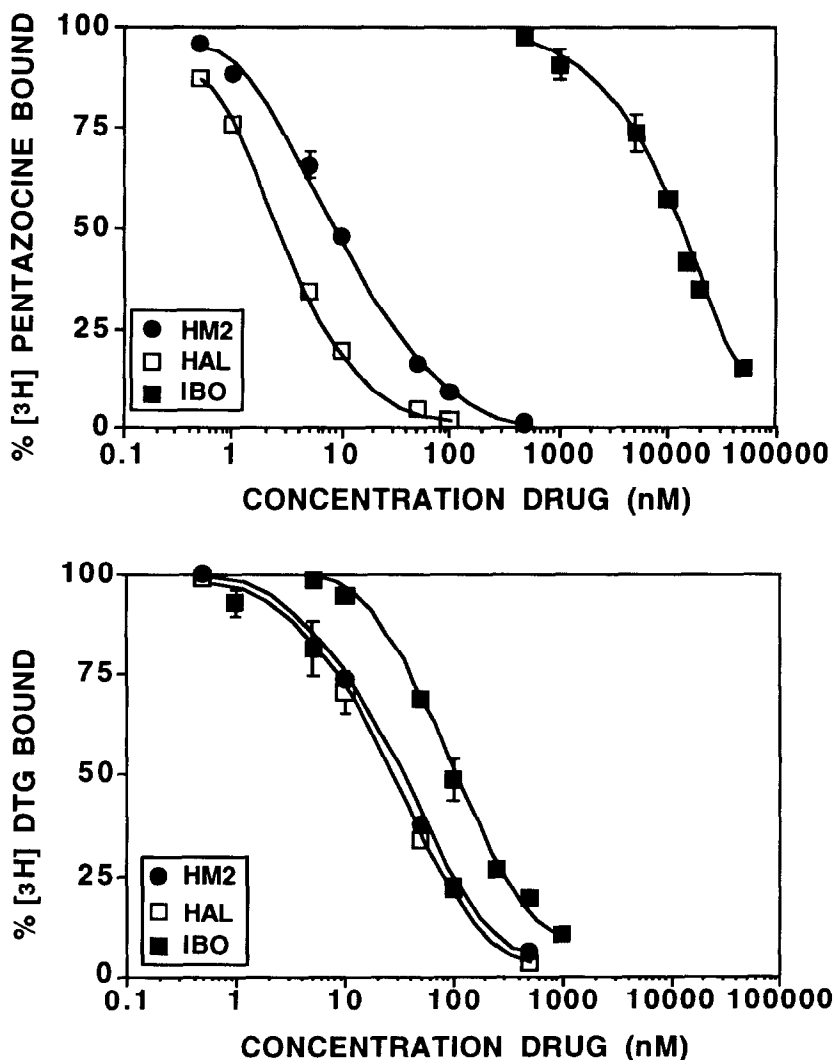


Fig. 1.

Displacement curves of [3 H](+)-pentazocine binding to σ_1 receptors (top) and [3 H]DTG binding to σ_2 receptors in rat liver (bottom) by haloperidol (HAL), haloperidol metabolite II (HM2) and ibogaine (IBO).

conditions of our assay reflected binding exclusively to the σ_1 receptor, competition curves with ibogaine in the σ_1 assay were also conducted with a 2 h and 4 h incubation time. A one-site fit was observed in both conditions (data not shown), and no significant difference in the K_i value of ibogaine was observed for the 2 h and 4 h incubation period (9,310 and 10,500 nM, respectively). Moreover, the concentration of [3 H](+)-pentazocine used in the σ_1 assays (3 nM) was too low to significantly bind to low affinity sites with a K_d value > 30 nM. This is why ibogaine, haloperidol, and haloperidol metabolite II displaced [3 H](+)-pentazocine with Hill slope values near unity, with the K_i value for haloperidol for the σ_1 receptor consistent with that reported elsewhere (15,16).

The σ_2 assay was initially conducted using rat liver membranes, which has been shown to be a rich tissue source of this receptor (15,16). The assay was conducted using [3 H]DTG in the presence of (+)pentazocine to mask σ_1 receptors, and the K_i value of ibogaine under these assay conditions was 90.4 nM. Although previous studies have shown that the σ_2 receptor expressed in rat liver has the same properties as that expressed in brain (16), a series of competition studies for the σ_2 receptor using guinea pig brain as the tissue source was also conducted in order to give further support that the behavioral effects of ibogaine may be mediated through an interaction with σ_2 receptors in the CNS. Although ibogaine possessed a slightly lower affinity for σ_2 receptors in guinea pig brain ($K_i = 250$ nM), its σ_2 affinity remained significantly higher than its σ_1 affinity. Moreover, a difference in binding affinity has been reported with other ligands when different tissues from the same species were used as the source of σ_2 receptors (16).

The results of this study clearly indicate that ibogaine has a relatively high affinity for σ_2 and a low affinity for σ_1 receptors. Although the affinity of ibogaine at sigma receptors was not as potent as our reference compounds (haloperidol and haloperidol metabolite II), our results are significant since the K_i values of 90.4 and 250 nM for σ_2 receptors represent the highest known affinity of ibogaine for any CNS receptor or target site reported to date. For example, ibogaine has a 6- to 30-fold higher affinity for σ_2 receptors than κ opiate receptors, a receptor previously identified as a putative site for the anti-addictive properties of ibogaine (12).

Although sigma receptors were originally classified as a subtype of the opiate receptors, it is now well recognized that sigma binding sites represent a distinct class of receptors located in both CNS and peripheral tissues (15). An understanding of the functional significance of sigma receptors has been hampered by the failure to confirm an endogenous ligand for this receptor. An additional complication is the failure to identify a signal transduction mechanism for sigma receptors, although there is some evidence suggesting σ_1 receptors may be directly coupled to guanine nucleotide binding proteins or indirectly coupled to phosphoinositol turnover through an interaction with cholinergic receptors (15). Less information is known about the signal transduction mechanism of σ_2 receptors. However, some evidence suggests that the σ_2 receptor is linked to potassium channels in NCB-20 cells (15,20). This limitation regarding the functional coupling of σ_2 receptors to a well-characterized signal transduction system prevents us from identifying whether ibogaine functions as an agonist or an antagonist at this site. In spite of the limited understanding in the pharmacology of σ_2 receptors, there is evidence suggesting that the behavioral effects of ibogaine may be mediated through an interaction with sigma receptors. Both ibogaine and sigma ligands block cocaine-induced locomotor activity (5,14) in rodents. In addition, the repeated administration of cocaine (21) and methamphetamine (22) have been shown to result in a persistent supersensitivity of sigma receptors in rats. Sigma receptors are also expressed in high densities in limbic regions of rat (23), guinea pig (24), rhesus macaque (25), and human brain (26); abused substances are well known for causing an increase in activity of the mesocorticolimbic dopaminergic system (27). Finally, the abnormal locomotor effects of ibogaine reported in a recent self-administration study in rats (9) are similar to that observed when sigma ligands are microinjected into the red nucleus of the same species (15). Additional studies are clearly needed in order to identify both the functional significance of the σ_2 receptor and its potential role in mediating the behavioral effects of ibogaine.

In conclusion, we have demonstrated that ibogaine has a relatively high affinity for σ_2 (90.4 or 250 nM) and a low affinity for σ_1 (9,310 nM) receptors. This affinity for σ_2 receptors is 6- to 30-fold higher than that previously reported for κ opiate receptors, a receptor identified as a putative site for the decreased drug craving properties of ibogaine (12). Our results clearly indicate that additional studies are needed in order to identify the functional significance of sigma receptors and whether or

not the behavioral effects of ibogaine are mediated through this site. In addition, the high selectivity for σ_2 vs. σ_1 receptors ($\sigma_2:\sigma_1$ ratio = 103 or 37.2) reported here indicates that ibogaine may be a suitable lead compound for further structure-activity studies aimed at preparing potent (i.e., low nanomolar affinity) σ_2 -selective ligands.

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